

# British American Tobacco Group Research & Development

## Method - Determination of selected volatiles in mainstream smoke

### 1 SCOPE OF APPLICATION

This method is applicable to the quantitative analysis of 5 selected volatile compounds (acrylonitrile, benzene, 1,3-butadiene, isoprene and toluene) in mainstream smoke samples using Gas Chromatography/Mass Spectrometry Detection.

### 2 NORMATIVE REFERENCES

- ISO 3308:2000 – Routine analytical cigarette smoking machine – definitions and standard conditions
- ISO 3402:1999 – Tobacco and tobacco products – atmospheres for conditioning and testing
- ISO 4387:2000 – Cigarettes - Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine
- ISO 8243:2006 – Cigarettes - Sampling

### 3 PRINCIPLE

Five conditioned cigarettes are smoked per port with 5 ports used for each cigarette sample (i.e. the samples are smoked as 5 replicates). Using a 20 port linear smoke engine the mainstream smoke is collected in two Dreschel type traps connected in series each containing methanol chilled by dry ice. The contents of the impingers are spiked with a known amount of internal standard (benzene-d<sub>6</sub>) and combined together. An aliquot of the combined sample is then vialled up and submitted for analysis by GC/MS in SIM mode.

### 4 HEALTH & SAFETY

Read and understand the Material Safety Data Sheets for the chemicals used in this method. Read and understand the method risk assessment. Ensure that you understand the hazards and follow control measures relevant to the operation of this method. All preparation of standards and extraction of samples must be performed in a fume cupboard.

### 5 REAGENTS AND MATERIALS

Dry ice (solid CO<sub>2</sub>)

Methanol - HPLC grade or equivalent

Benzene-d<sub>6</sub> ≥ 99.6%, C<sub>6</sub>D<sub>6</sub>

1,3-Butadiene in methanol certified at 200ng/μL

Certified mixed standard of isoprene, acrylonitrile, benzene and toluene

### 6 APPARATUS

Gas Chromatograph fitted with a liquid autosampler and mass spectrometry detector

Soap bubble manometer to measure puff volume

20 port linear smoke engine – adapted for use with liquid traps

Analytical balance capable of measuring to at least four decimal places

44 mm Cambridge filter holders (screw type) and 44mm pads

Nalgene or Tygon FDA/USPVI grade metric 180 PVC tubing

Quickfit® tubes and Dreschel heads

Normal laboratory glassware including volumetric flasks

Range of calibrated variable volume positive displacement pipettes  
2 mL amber GC vials with crimp top caps (Teflon coated)  
Dewar flasks with holders  
230 mm Pasteur pipettes  
Solvent dispenser capable of dispensing 10 mL ± 0.2 mL  
UV Spectrophotometer

## 7 PRELIMINARY SAMPLE PREPARATION

Cigarettes should be conditioned according to normal procedures (ISO 3402:1999). Unless specifically requested cigarettes are not subjected to any selection criteria other than the rejection of any obviously defective or damaged cigarettes. Butt marking is as specified in ISO 4387:2000 unless otherwise requested.

## 8 ANALYTICAL PROCEDURE –SOLUTION PREPARATION

### 8.1 Extracting solution

The extracting solution is HPLC grade methanol. Pour this into the solvent dispenser in readiness for trap preparation ensuring it is free from air bubbles.

### 8.2 Preparation of internal standard

#### 8.2.1 Internal Standard Stock solution

Prepare a stock solution of benzene-d<sub>6</sub> by transferring the contents of a 0.5 mL ampoule to a 10 mL volumetric flask. Fill to the mark with methanol and mix well. This stock is stable for 2 weeks if stored in the freezer when not in use.

#### 8.2.2 Internal Standard Spiking solution

Prepare the internal standard spiking solution by diluting 4 mL of the stock (8.2.1) to 100 mL with methanol and mixing well. This will give a final concentration of approximately 1.76 mg/mL.

This solution is stable for 2 weeks if stored in the freezer when not in use.

### 8.3 Calibration standards

#### 8.3.1 Isoprene, acrylonitrile, benzene and toluene stock standards

Open two 1 mL ampoules of the certified volatiles standard and transfer to a 7 mL amber vial. This mixture is referred to as the primary calibration stock (S1) and has the following concentrations:

Table 1 – Concentration of certified volatiles mixture (S1)

Component	Conc. (µg /mL)
Isoprene	4,086
Acrylonitrile	162
Benzene	703
Toluene	1,040

Dilute the S1 solution by adding 100 µL to a 20 mL volumetric flask and making up to the mark with methanol (Table 2). This is the S2 solution and should be made with each preparation of the standards.

Table 2– Preparation of secondary calibration stock (S2)

Stock	Component	S1 Volume (mL)	Final Volume (mL)	Conc. (µg/mL)
S2	Isoprene	0.1	20	20.43
	Acrylonitrile			0.81
	Benzene			3.51
	Toluene			5.20

The 1,3-Butadiene solutions (10.2.3) should only be prepared when S1 and S2 solutions have been prepared.

### 8.3.2 Primary 1,3 butadiene stock standard

The preparation of the 1,3-butadiene standards involves the use of a certified standard at 200 ng/µL. Due to the volatility of this compound it is good practice to check the concentration before preparing the calibration.

#### 8.3.2.1 Concentration check

Open seven 1 mL ampoules of 1,3-butadiene in methanol in order to prepare the calibration and store in an appropriate vial. Pipette 0.2 mL of this solution into a 20 mL volumetric flask containing methanol and make up to the mark and mix well.

Measure the absorbance of the solution against a methanol blank on the spectrophotometer at 217 nm. Make a record of the absorbance which should be over 0.4.

Calculate the concentration of the certified solution by using the following calculation:

$$\text{Conc. (}\mu\text{g/mL)} = \frac{A}{20\,893\text{ L/mole}} \times 54\text{ g/mole} \times \frac{1000\text{ mg/g}}{1000\text{ mL/L}} \times \frac{20\text{ mL}}{0.2\text{ mL}} \times 1000\ \mu\text{g/mg}$$

A = Absorbance

### 8.3.3 Secondary 1,3 butadiene stock standard

The certified 1,3-butadiene standard at 200 ng/µL is referred to as the SB1 stock. The SB2 stock is prepared from this by taking 500 µL of SB1 and adding to a 5 mL volumetric flask and making up to the mark with methanol.

### 8.3.4 Working calibration solutions

Using the SB1 and SB2 solutions prepare the combined vapour phase working standards as described in Table 3. This will give the final concentrations shown in Table 4.

**Table 3 – Preparation summary of working calibration solutions**

Calibration Level	Volume S1/S2 (µL)	Volume SB1/SB2 (µL)	Volume Int. Std. (µL)	Final Volume (mL)	Solvent
1	100 S2	80 SB2	200	20	Methanol
2	1000 S2	160 SB2	200	20	Methanol
3	40 S1	160 SB1	200	20	Methanol
4	80 S1	320 SB1	200	20	Methanol
5	200 S1	880 SB1	200	20	Methanol
6	400 S1	1760 SB1	200	20	Methanol
7	800 S1	2500 SB1	200	20	Methanol

**Table 4 – Working calibration concentrations**

CAL Level	Concentration (µg /mL)				
	1,3-Butadiene	Isoprene	Acrylonitrile	Benzene	Toluene
1	0.080	0.102	0.0041	0.018	0.026
2	0.016	1.022	0.0405	0.176	0.260
3	1.600	8.172	0.324	1.406	2.081
4	3.200	16.344	0.648	2.812	4.161
5	8.800	40.860	1.620	7.029	10.403
6	17.600	81.720	3.240	14.058	20.806
7	25.000	163.44	6.480	28.116	41.612

After preparing the standards allow them to cool in a freezer for at least 20 minutes before cold vialling. Using pasteur pipettes transfer each calibration solution to an autosampler vial and fill to the neck. Crimp with a teflon coated vial cap. Each vial is used once and then discarded. Vials are stable for 10 days if stored in a freezer when not in use.

**Note** Concentrations for 1,3-butadiene are based on the certified amount of 200ng/µL. When entering calibration data in Chemstation ensure that all analytes are given a 1/x weighting.

#### 8.4 Calibration Check Solution

Prepare the calibration check solution in methanol as described in Table 5 using the same methodology as for preparing the calibration standards. Ensure that different batches of components are used to the calibration standards. For the preparation of the 1,3-butadiene component of the Calibration Check Solution it is acceptable to use the same solution that is used for preparing the calibration.

**Table 5 – Preparation summary of Calibration Check solution**

Volume S1 (µL)	Volume SB1 (µL)	Volume Int. Std. (µL)	Final Volume (mL)	Concentration (µg /mL)				
				1,3-Butadiene	Isoprene	Acrylonitrile	Benzene	Toluene
100	400	500	50	1.600	8.172	0.324	1.406	2.081

After preparing the standards allow them to cool in a freezer for at least 20 minutes before cold vialling. Using pasteur pipettes transfer each calibration solution to an autosampler vial and fill to the neck. Crimp with a teflon coated vial cap. Each vial is used once and then discarded. Unused vials are stable for 10 days if stored in the freezer when not in use.

## 9 ANALYTICAL PROCEDURE – SAMPLE PREPARATION

### 9.1 Sample Collection – Preparation of liquid traps

This method utilises two liquid impingers attached in series to the back of each smoking port. This is a deviation from the ISO Method 3308:2000.

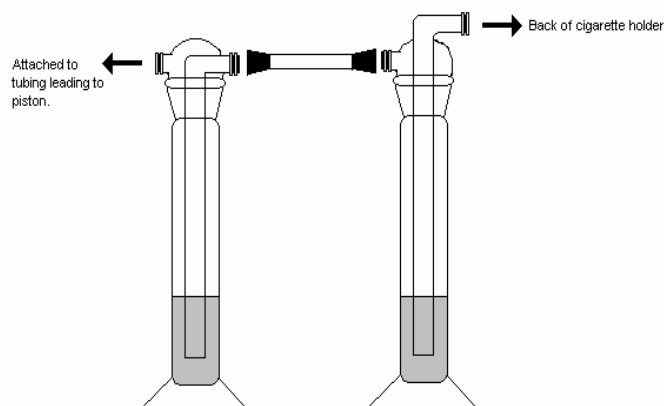
Prior to smoking allow the smoking machine to warm up for 30 minutes and measure airflow in ports 1, 10 and 20 with an airflow probe. Individual port readings should be 200mm/sec ( $\pm 50$ mm/sec) and the average airflow across the measured ports should be 200mm/sec ( $\pm 30$ mm/sec).

Before smoking weigh 44mm Cambridge filter pads (CFP) and holders record the weights. Check the methanol dispenser with a measuring cylinder to ensure it dispenses 10 mL ( $\pm 0.2$  mL) of methanol.

### 9.2 Assembly of trapping system

Assemble the vapour phase trapping system behind the cigarette holder as shown below, with each trap containing 10mL methanol delivered from a pre-set dispenser.

Assemble the smoke collection traps in Dewar flasks packed with dry ice. Ensure that the dry ice is packed to at least half the height of each trap.



**N.B. Take great care to ensure that the traps are connected correctly otherwise instrument damage can occur.**

Before commencing the smoke engine checks it is vital that the traps are chilled by the dry ice as this will affect the puff volume readings. Allow **at least 10 minutes** for the methanol to equilibrate to temperature before proceeding.

### 9.3 Sample Collection

Check the system for leaks by taking a puff and ensuring liquid does not siphon back up the impinger tube. Check the puff volumes with the traps in position and adjust if necessary. Puff volumes should be 35mL  $\pm 0.3$ mL (for ISO smoking).

Smoke 5 conditioned cigarettes through each trapping system. One clearing puff is taken between each cigarette run, and a further four clearing puffs are taken at the end of the run to ensure any residual smoke has been collected in the trapping solution. Record the number of lit puffs and dismantle the traps from the smoke machine. Reweigh the CFPs and holders in order to calculate TPM values. Record this data.

## 9.4 Sample Extraction

Add 101 µL of working internal standard into each of the traps. The two impingers are then combined and an aliquot of approximately 1mL is added to a labelled GC vial and capped.

## 10 ANALYTICAL PROCEDURE – INSTRUMENTAL ANALYSIS

### 10.1 Instrument Set Up Parameters

Analysis is performed on an Agilent 6890 fitted with a liquid autosampler and Agilent 5973 Mass Selective Detector (MSD).

GC Column: RTX-VMS column (length 30 m, 0.32 mm i.d., 1.8µm film thickness).

1.0 µL Split injection: 10:1 at 10 psi

Injector temperature: 220°C

Constant flow: 3.4 mL/min

GC Oven temperature programme:

	40°C	Hold for 5.0 minutes
20°/min	225	Hold 1 minute
Total run time (minutes)		15.25

MSD Conditions:

Source Temperature: 230°C

MS Quad: 150°C

Ionisation Mode: EI+

Solvent Delay: 0.8 minutes

Table 6 – Summary of target and qualifier ions.

Component	Target (m/z)	Qualifiers (m/z)	MS Acquisition Window no.	MS Window start time (mins)	RT (mins)
1,3-Butadiene	54(100%)	53 (54-79%), 51 (28-42%)	1	0.80	1.08
Isoprene	67 (100%)	68 (56-84%), 53 (48-70%)	2	1.30	1.53
Acrylonitrile	53 (100%)	52 (56-84%), 26 (40-60%)	3	2.20	2.78
Benzene	78 (100%)	77 (16-24%), 51 (10-24%)	4	3.22	4.36
D <sub>6</sub> -Benzene	84 (100%)	82 (12-18%), 42 (5-9%)			
Toluene	91 (100%)	92 (48-72%), 65 (8-12%)	5	6.00	7.18

All of the above ions have a dwell time of 40 milliseconds.

Standards and samples are quantified on the target ions stated above. The qualifier ion ratios should be used to confirm that the peaks in the standards are the correct compounds. For each component a calibration plot of standard concentration ratio Vs peak area ratio is constructed.

Standards and samples are quantified on the target ions stated above. The qualifier ion ratios are used to confirm that the peaks in the standards are correctly identified. In addition the ion ratios of the sample peaks should be within 20% of the standard ion ratios in that run.

### 10.2 System Suitability Criteria

#### 10.2.1 MS tuning

Tune MS weekly, or if system has been vented. Check the following criteria are met on the tune report:

air and water peaks <10%  
EM volts 1000 – 3000  
Ion ratio of 502:219:69 is 10:4:1 respectively.  
Peak width approx. 0.6 ( $\pm$  10%)

### **10.2.2 Peak resolution check**

Open a chromatogram of the control sample and use the instrument software to integrate the acrylonitrile peak and the interfering peak to the right. The valley should be greater than 80%.

If the valley is <80% investigate the problem before further analysis takes place.

Record the value in the maintenance log.

### **10.2.3 Peak shape check**

Open a chromatogram of the Calibration check solution, and use the instrument software to assess peak shape.

The peak of interest is isoprene.

The value for tailing should be <1.3. If the result is greater than 1.3 the problem should be investigated before further analysis. Record the value in the maintenance log.

### **10.2.4 Ion ratio check**

Check ion ratios are within limits in section 10.1

### **10.2.5 Calibration linearity**

The R<sup>2</sup> value of each of the calibration graphs must be >0.99.

## **10.3 Run Order**

Start the run with a vial of blank methanol, then:

Standards in ascending order (1-7)

Calibration Check Solution

Control cigarette 1

Smoked samples (5 replicates)

Control cigarette 2

Smoked samples (5 replicates)

Calibration Check Solution

Repeat the above steps until all samples have been analysed

Finish on a Calibration Check Solution

## **11 CALCULATIONS**

Using the instrument software plot a calibration graph of calibration standards concentration against peak area ratio, without forcing the line through zero for each of the compounds of interest.

Peak data is measured using selected ions for each component.

Standards and samples are quantified on the target ions stated in Table 6. The qualifier ion ratios should be used to confirm that the peaks in the standards are the correct compounds.

For each component a calibration plot of standard concentration ratio against internal standard Vs peak area ratio is constructed.

Please note:

Agilent Chemstation – data analysis software performs the following calculation (for µg/mL), it is here for information only.

All calibration plots should be linear with an R<sup>2</sup> of 0.99 or better using a 1/x weighting. The slope and intercept of the calibration line are used to convert peak area ratios for samples to results in µg/cig as follows:

$$\text{Component in } \mu\text{g/mL} = \frac{\text{PAR} - C}{M} \times \text{ISTD Conc } (\mu\text{g/mL})$$

$$\text{Component in } \mu\text{g/cig} = \frac{\text{Component in } \mu\text{g/mL} \times 20.2}{5}$$

Where :

- PAR - component peak area ratio
- M - slope of linear calibration equation
- C - intercept on the Y axis of the linear calibration equation
- ISTD - internal standard
- Component µg/cig - concentration of component in µg/cig

## 12 PRECISION AND REPORTING LIMITS

The lower reporting limit is defined by the concentration of the lowest calibration standard, and equates to the concentrations in the table below.

Table 7 – Summary of Minimum Reporting Limits

Concentration	1,3 butadiene	Isoprene	Acrylonitrile	Benzene	Toluene
µg/cig	0.32	0.41	0.16	0.07	0.11

## 13 QUALITY ASSURANCE AND CONTROL

Control charts of the Calibration Check Solution and the reference cigarette are maintained to allow inspection of the method performance.

## 14 SPECIAL CASES

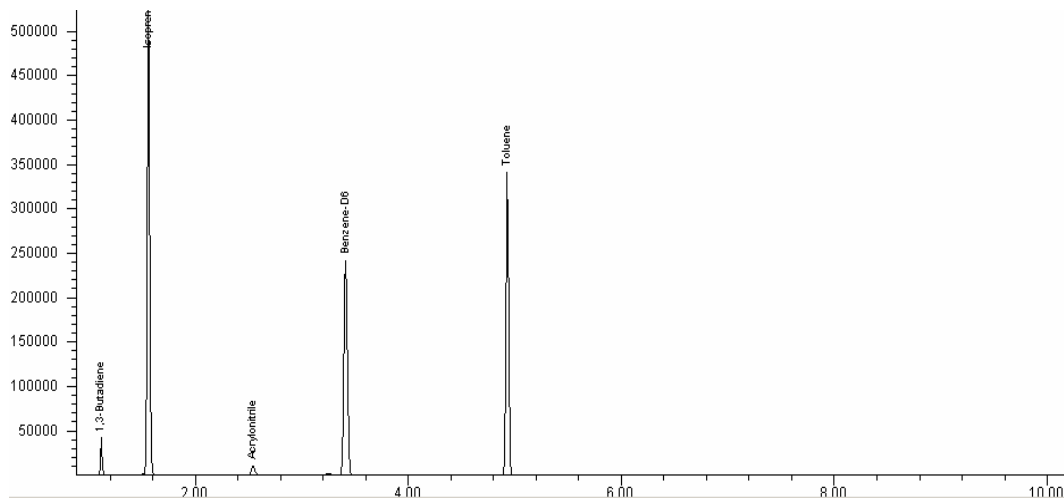
Under more intense smoking regimes, the number of cigarettes per smoking run may need to be reduced in order to avoid smoke breakthrough on the Cambridge filter pad.

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# APPENDIX A SAMPLE CHROMATOGRAMS

## Standard



## Smoke sample – Reference Cigarette

